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(71) Applicant: LIGAND PHARMACEUTICALS INCORPORATED [US/US]; 9393 Towne Centre Drive, San Diego, CA 92121 (US).

(72) Inventor: MUKHERJEE, Ranjan; 11341 Avenida De Los Lobos, San Diego, CA 92127 (US).

(74) Agents: WARBURG, Richard, J. et al.; Lyon & Lyon L.L.P., Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071-2066 (US).

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(54) Title: PPAR GAMMA ANTAGONISTS FOR TREATING OBESITY

(57) Abstract

The present invention relates to a method of treating obesity, diabetes and other metabolic disorders in a mammal by administering to the mammal a pharmaceutical composition containing a compound that antagonizes the activity of PPARγ protein, or reduces the expression of PPARγ protein. This invention also features methods of screening for compounds for treating obesity, diabetes and other metabolic disorders.

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# PPAR GAMMA ANTAGONISTS FOR TREATING OBESITY

## FIELD OF THE INVENTION

This invention relates to compounds and methods for treating obesity and other metabolic disorders. This invention also relates to screening for compounds having the aforesaid therapeutic effects.

## BACKGROUND OF THE INVENTION

Obesity is usually defined as a body weight more than 20% in excess of the ideal body weight. Obesity is associated with an increased risk for cardiovascular disease, noninsulin dependent diabetes mellitus (NIDDM), hypertension, coronary artery disease and an increased mortality rate (see Grundy et al., Disease-a-Month 36:645-696, 1990). Treatment for obesity includes diet, exercise and surgery (Leibel, R.L. et al., New England Journal of Medicine 332:621-628, 1995).

Obesity is related to abnormal number or function of adipocytes. Adipocytes store energy in the form of triglcerides during periods of nutritional abundance and release it in the form of free fatty acids at times of nutritional deprivation.

The function of adipocytes is related to peroxisomes, which are subcellular organelles found in animals and plants. Peroxisomes contain enzymes for cholesterol and lipid metabolism and respiration.

A variety of chemical agents called peroxisome proliferators induce the proliferation of peroxisomes and increase the capacity of peroxisomes to metabolize fatty acids

via increased expression of the enzymes required for the β-oxidation cycle. Peroxisome proliferators include unsaturated fatty acids, hypolipidemic drugs (Reddy, J. K., and Azarnoff, D. L., Nature 283:397-398, 1980), herbicides, leukotriene antagonists, and plasticizers (for a review, see Green, S., Biochem. Pharmacol. 43:393-400, 1992). Hypolipidemic drugs such as clofibrates have been found to lower triglycerides and cholesterol levels in plasma and to be beneficial in the prevention of ischemic heart disease in individuals with elevated levels of cholesterol (Havel, R.J. and Kane, J.P., Ann. Rev. Pharmac. 13:287-308, 1973). However, fibrate hypolipidemic drugs are also rodent hepatocarcinogens (Reddy, J. K., et al., Br. J. Cancer 40:476-482, 1979; Reddy, J. K., et al., Nature 283:397-398, 1980).

Peroxisome proliferator activated receptors (PPARs) have been isolated and cloned from various species (Isseman, et al. Nature 347:645-650, 1990; Dreyer, et al., Cell 68:879-887, 1992; Gottlicher, et al. Proc. Natl. Acad. Sci. USA. 89:4653-4657, 1992; Sher, et al. Biochemistry 32:5598-5604, 1993; and Schmidt, et al. Mol. Endocrinol. 6:1634-16414-8, 1992; Tontonoz, *et al*. Genes & Development 8:1224-1234, 1994; Kliewer, *et al*. Proc. Natl. Acad. Sci. 91:7355-7359, 1994; Chen, *et al*. Biochem. and Biophy. Res. Com. 196:671-677, 1993). The peroxisome proliferator activated receptor subtypes are members of the intracellular receptor superfamily.

A subtype of peroxisome proliferator activated receptors, PPARγ, plays a key role in adipocyte differentiation. Two isoforms of PPARγ (PPARγ1 and PPARγ2

that differ by 30 amino acids at the N-terminus) have been identified in mice (Tontonoz, *et al.* Genes & Development 8:1224-1234, 1994). PPARγ2 is expressed at high levels specifically in adipose tissue and is induced early in the course of differentiation of 3T3-L1 preadipocytes to adipocytes. Overexpression and activation of PPARγ protein stimulates adipose conversion in cultured fibroblasts (Tontonoz, *et al.* Cell 79:1147-1156, 1994, not admitted to be prior art). Activation of PPARγ is sufficient to turn on the entire program of adipocyte differentiation (Lehmann, *et al.* J. Biol. Chemistry 270:12953-12956 (1995), not admitted to be prior art).

## SUMMARY OF THE INVENTION

This invention relates to altering adipocyte differentiation and treating obesity by antagonizing the activity of PPARγ protein or reducing the expression of PPARγ protein.

Expansion of adipose mass requires *de novo* differentiation from precursor cells (Ailhaud, *et al.* Annu. Rev. Nutr. 12:207-233, 1992). Over expansion and accumulation of adipose tissue and other disorders of adipose tissue contribute to obesity, diabetes, lipoprotein defects, hypertension, hyperlipidemia, hypercholesteremia, hyperlipoproteinemia and other metabolic diseases or disorders.

This invention discloses that compounds which antagonize the activity of PPARγ protein and/or reduce the expression of PPARγ protein are effective in blocking the

differentiation of the precursor cells to adipose tissue and is therefore useful in the treatment of obesity, diabetes, lipoprotein defects, hypertension, hyperlipidemia, hyperchloesterolemia, hyperlipoproteinemia and other metabolic diseases or disorders.

Thus, in a first aspect, this invention features a method of treating obesity, diabetes, lipoprotein defects, hypertension, hyperlipidemia, hyperchloesterolemia , hyperlipoproteinemia and other metabolic diseases or disorders in a mammal, including, but not limited to, a human, by administering to the mammal a pharmaceutical composition containing a compound which antagonizes the activity of PPARγ protein or reduces the expression of PPARγ protein.

By "activating" or "antagonizing" is respectively meant increasing or decreasing the activity of a PPAR protein in a dosage dependent manner. An activating or antagonizing agent is respectively capable of increasing or decreasing the biochemical activity of a protein by two-fold (preferably by five-fold, more preferably by ten-fold, and even more preferably by a hundred-fold). Such activity includes, but is not limited to, the transcription activation activity of a PPAR protein. PPARγ is known to form heterodimer with other intracellular proteins, including, but not limited to, thyroid hormone receptor (TR), liver enriched X receptor (LXR) and RXR. These heterodimers modulate the transcription activity of genes involved in adipocyte differentiation and other metabolic pathways. A compound of this invention may selectively antagonize a particular PPARγ heterodimer or a number of different PPARγ heterodimers. By antagonizing the

transcription activity of one or more PPARγ heterodimers, the compounds of this invention modulate adipocyte differentiation, obesity, and related symptoms.

By "reducing the expression of PPARγ protein" is meant decreasing the level of PPARγ protein by two-fold (preferably by five-fold, more preferably by ten-fold, and even more preferably by a hundred-fold).

In a second aspect, this invention features a method of treating obesity, diabetes, lipoprotein defects, hypertension, hyperlipidemia, hyperchloesterolemia, hyperlipoproteinemia and other metabolic diseases or disorders in a mammal, including, but not limited to, a human, by administering to the mammal a pharmaceutical composition containing a compound which reduces the expression of PPARγ protein or antagonizes the activity of PPARγ protein.

In a third aspect, this invention features a method for screening compounds for treating obesity, diabetes, lipoprotein defects, hypertension, hyperlipidemia, hyperchloesterolemia, hyperlipoproteinemia and other metabolic diseases or disorders by contacting a test compound with an adipocyte or preadipocyte cell, then measuring or detecting its effect in lowering the level of triglyceride, lipoprotein lipase, fatty acid synthetase, aP2, adipsin, or PEPCK as an indication of its therapeutic utility.

By "preadipocyte" is meant a cell that can be induced to differentiate into an adipocyte by chemicals, such as, but not limited to, dexamethasone, insulin, 3-Isobutyl-1-Methyl-Xanthine (IBMX), BRL 49653, thiazolidinedione, or any

combinations of the above. 3T3-L1 cell is an exapmle of preadipocyte.

In preferred embodiments, compounds that antagonize the activity of PPARγ protein, or reduce the expression of PPARγ protein are selected for testing in the assay. In further preferred embodiments, selected candidate compounds are those that reduce the transcription regulation activity of a PPARγ heterodimer, including, but not limited to, PPARγ/LXR heterodimer, PPARγ/TR heterodimer and PPARγ/RXR heterodimer.

The compounds identified by these methods are particularly useful in the treatment of diseases and pathological conditions affected by the activity of PPARγ protein, including, without limitation, obesity, diabetes, lipoprotein defects, hypertension, hyperlipidemia, hyperchloesterolemia, hyperlipoproteinemia and other metabolic diseases or disorders.

Such methods are particularly usful for the identification of agents of low molecular weight (less than 10,000 daltons, preferably less than 5,000, and most preferably less than 1,000) which can be readily formulated as useful therapeutic agents. Steroids and steroid analogues exemplify agents which can be tested.

The following classes of chemicals compounds are candidate compounds: thiazolidinediones (*see* T.M. Willson et al. (1996), J. Med. Chem. 39:665-668), eicosanoids (*see* K. Yu et al. (1995) J. Biol. Chem. 41:23975-23983), leukotrienes, retinoids (*see* S. Canan Koch et al. (1996) J. Med. Chem. 39:3229-3234), fibrates (*see* A. Lozada et al. (1994) Pharmac.

Ther. 63:163-176), and prostaglandins (*see* B. Forman et al. (1995) Cell 83:803-812).

Once isolated, a candidate agent can be put in pharmaceutically acceptable formulations, such as those described in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co., Easton, PA (1990), incorporated by reference herein, and used for specific treatment of diseases and pathological conditions with little or no effect on healthy tissues.

In another aspect, this invention features a pharmaceutical composition capable of treating obesity, diabetes, lipoprotein defects, hypertension, hyperlipidemia, hyperchloesterolemia, hyperlipoproteinemia and other metabolic diseases or disorders. The composition is held within a container which includes a label stating to the effect that the composition is approved by the FDA in the United States (or other equivalent labels in other countries) for treatment of a disease or condition selected from the group consisting of obesity, diabetes, cardiovascular diseases, coronary diseases, hypertension, hyperlipidemia, hyperchloesterolemia and hyperlipoproteinemia. Such a container will provide sufficient compound to allow a therapeutically effective amount to be administered in a therapeutically effective manner to a patient.

One of ordinary skill in the art can identify a mammal for treatment by diagnosing disorders of adipose tissue in the mammal subject. Pharmaceutical compositions may be administered to the mammal subject by methods known to one

skilled in the art, including, but not limited to, those disclosed in the preferred embodiments.

The present invention also includes pharmaceutically acceptable compositions prepared for storage and subsequent administration which include a therapeutically effective amount of an above-described compound in a pharmaceutically acceptable carrier or diluent.

By "therapeutically effective amount" is meant an amount of a pharmaceutical composition having a therapeutically relevant effect. A therapeutically relevant effect relieves to some extent one or more symptoms of the disease or condition in the patient; or returns to normal either partially or completely one or more physiological or biochemical parameters associated with or causative of the disease or condition.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

### Screening for compounds for treating obesity

Disclosed below are exemplary assays to screen for compounds useful for treating obesity, diabetes and other metabolic disorders. Any candidate compound can be tested by these assays. Other assays known to those skilled in the art may also be used, including, but not limited to, those disclosed or identified in U.S. application 08/484,487, entitled "Human Peroxisome Proliferator Activated Receptor γ" by Mukherjee, incorporated by reference herein.

The host cells used in the screening assay herein generally are mammalian cells, and preferably are human cell lines.

Mammalian cells of choice are preadipocyte or adipocyte, e.g., 3T3-L1 or 3T3 F422A or *ob* 1771 (uninduced or induced to differentiate). In a preferred embodiment, isolated rat primary adipocytes are used as a model assay system.

Cell systems other than mammalian may also be used in the screening assays, such as Drosophila (SL-2, Kc or others) and yeast strains (permeabilized or not) such as S. cerevisiae or S. pombe.

Animals such as mice can be used both as a primary screening vehicle in which compounds can be administered and parameters such as feeding, weight, levels of glucose, insulin, triglyceride, Lipoprotein lipase and PPARγ protein or mRNA production can be measured along with other appropriate controls to effectively assess the changes in expression of PPARγ protein or mRNA as well as a means of corroborating primary compound positives.

A reporter gene responsive to PPAR activation could be introduced into animals utilizing the standard transgenic practice or adenovirus drag technology in which the target DNA is admixed with poly-L-lysine and/or transferrin or asialoglycoprotein modified adenovirus and injected i.v. into the animal, resulting in expression of the foreign DNA (Wu et al., JBC 266:14338-14342, 1991; Yanow et al.1993, PNAS 90:2122-2126). In a preferred embodiment, adenovirus carrying the exogenous DNA can be injected directly into fat deposits

of mice, rats or other species as has been done previously in brain (Davidson, Nature Genetics 3:219) (Science 259:988), muscle (Quantin PNAS 89:2581) (Statford-Perricaudet J. Clin. Invest. 90:626), and tumors (Tang, Johnston and Carbone in a recent issue of Cancer Gene Therapy). These animal model assay systems are also useful in secondary characterization and study of compounds found to treat obesity identified in other assays.

Example 1. Screening for compounds that antagonize the activity of PPARγ protein

CV-1 cells are cultured in DMEM with 10% FBS. Cells are plated in Costar 96 well plates at a density of 5000 cells per well the day before they are transfected with plasmids. CV-1 cells are transfected with pCMXmPPARγ (Kliewer, *et al.* Proc. Natl. Acad. Sci. 91:7355-7359 (1994)) and the PPREA3-tk-LUC reporter (Kliewer, *et al.* Nature 358:771-774 (1992)).

Transient transfections are performed by the calcium phosphate precipitation method. Plasmids are mixed in the following ratios: 1μg pCMXmPPARγ expression vector, 9μg pGEM vector, 5μg pRS-β-Gal2, and 5μg of pPREA3-tk-LUC luciferase reporter. Each well receives 100 ng of precipitated DNA mix which is left on the plate for 5-6 hours. Plates are then washed with PBS and fresh media containing 10% charcoal-absorbed FBS and drug is added.

Cells are incubated with drugs for 40 hours. The cells are then lysed and analyzed for luciferase and β-gal activity. Normalized response is the luciferase value divided

by the β-gal activity for each well. Transfections are done in triplicate and each data point is the average from the three wells. Each experiment is performed at least three times. BRL 49653 (Lehmann, *et al.* J. Biol. Chemistry 270:12953-12956, 1995) is an anti-diabetic agent which induces the activity of PPARγ protein. BRL 49653 is added to a final concentration of 320 nM. A candidate PPARγ antagonist is added to the assay.

In transiently transfected CV-1 cells, a PPARγ dependent transcriptional activation (about 30-60 fold) is observed with 320 nM BRL 49653.

A candidate compound which antagonizes the ability of BRL 49653 to activate PPARγ in a dose dependent manner is selected as a PPARγ antagonist.

Example 2. Staining Assay

A candidate compound can be examined for its effects to prevent the differentiation of 3T3-L1 pre-adipocyte cells into adipocytes.

3T3-L1 cells have been used as a model system to study adipocyte biology and can be induced to differentiate into adipocytes with dexamethasone, insulin and IBMX (i.e. DIM) (Tontonoz).

3T3-L1 cells (ATCC) are maintained in DMEM supplemented with 2 mM L-glutamine, 55 mg/ml gentamicine (BioWhittaker) and 10% calf serum (regular media). Cells are plated at 80% confluency and induced to differentiate 2 days after reaching confluency by replacing the media with differentiation media (DMEM plus 10% fetal calf serum, 1.6 μM

insulin, 1$\mu$M dexamethasone and 500$\mu$M 3-Isobutyl-1-Methyl-Xanthine). After 3 days the media is changed to DMEM plus 10% fetal calf serum and insulin. Three days later the cells are fixed in formalin and cellular lipids visualized by Oil Red O staining (S.W. Thompson, R.D. Hunt & Charles C. Thomas, Selected Histochemical and Histopathological Methods, Springfield, Illinois, 1966, p. 330).

3T3-L1 cells are maintained in regular media or in differentiation media. A candidate compound is added with the differentiation media. Cells are fixed and stained with Oil Red O. A reduced adipocyte staining shows that the candidate compound block differentiation of 3T3-L1 preadipocytes into adipocytes.

Although not as active as DIM, BRL 49653 also induces the differentiation of 3T3-L1 cells into adipocytes and can be used to screen for a compound which blocks the inducement of adipocyte differentiation.

Example 3. Triglyceride assay

A candidate compound can also be examined for its effects on the triglyceride level of adipocyte cells. 3T3-L1 cells are differentiated in differentiation medium as described above in 96 well plates (Costar). They are lysed in 50 $\mu$l PBS containing 0.1% NP40 for 10 minutes at room temperature. 5 $\mu$l is used to measure protein concentration by the method of Bradford (Bio-rad). The remaining 45 $\mu$l is used to measure the triglyceride (GPO-Trinder) reagent (Sigma). The optical density measured at 540 nanometers is normalized

to the protein content. A compound which lowers the triglyceride level in a dose dependent manner is selected.

Example 4. Screening for compounds that reduce the expression of PPARγ protein with Northern blot assay

Expression of adipocyte specific genes in a candidate compound treated cells can be examined by Northern blot analysis.

3T3-1 cells are differentiated by DIM inducement as described above. Poly(A)+ RNA is isolated using PolyATract system (Promega). PPARγ (Kliewer), LPL (Auwerx, Biochemistry 19:2651-2655, 1988) and actin (Clonetech) is labeled by random priming (Stratagene).

Northern blot analysis is performed with 2μg of poly (A)+ RNA from undifferentiated cells, differentiated cells or from cells grown in differentiation media plus the candidate compound and hybridized to $^{32}P$ labeled PPARγ, lipoprotein lipase probes.

Both PPARγ and lipoprotein lipase (LPL) are dramatically induced during 3T3-L1 differentiation. A candidate compound which significantly reduces PPARγ and lipoprotein lipase (LPL) is selected. A compound that does not significantly change actin expression is preferred for its specific activity on the expression of PPARγ protein and an adipocyte specific marker, LPL, and the morphology of 3T3-L1 cells.

Example 5. Screening for PPARγ antagonist in yeast or animals

To screen for PPARγ antagonist in yeast, PPARγ is inserted into a suitable yeast vector and transferred into yeast and expressed. A reporter gene such as β-galactosidase, luciferase, secreted alkaline phosphatase, CAT or other reporter systems is constructed to be responsive to PPARγ to measure the effects of compounds added to the yeast culture. BRL 49653 or any other PPARγ agonist is used to activate PPARγ. Compounds that antagonize the PPARγ activity are selected.

To screen for PPARγ antagonist in animals, animals (e.g., mice or rats) are treated with BRL 49653 to increase their weights to a level higher than control (S.W. Thompson, R.D. Hunt & Charles C. Thomas, Selected Histochemical and Histopathological Methods, Springfield, Illinois, 1966, p. 330). Compounds that can reverse the weight gain in animals are selected.

Pharmaceutical Formulations and Modes of Administration

The particular compound that affects the disorders or conditions of interest can be administered to a patient either by themselves, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s). In treating a patient exhibiting a disorder of interest, a therapeutically effective amount of an agent or agents is administered. A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms or a prolongation of survival in a patient.

In addition, the molecules tested can be used to determine the structural features that enable them to treat obesity, diabetes and other metabolic disorders, and thus to select molecules useful in this invention. Those skilled in the art will know how to design drugs from lead molecules, using techniques such as those disclosed in PCT publication WO 94/18959, incorporated by reference herein.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the $LD_{50}$ (the dose lethal to 50% of the population) and the $ED_{50}$ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio $LD_{50}/ED_{50}$. Compounds which exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the $ED_{50}$ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the $IC_{50}$ as determined in cell culture (i.e., the concentration of the test compound which achieves a half-maximal disruption of the protein

complex, or a half-maximal inhibition of the cellular level and/or activity of a complex component). Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by HPLC.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g. Fingl et al., in The Pharmacological Basis of Therapeutics, 1975, Ch. 1 p. 1). It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administrated dose in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

Depending on the specific conditions being treated, such agents may be formulated and administered systemically or locally. Techniques for formulation and administration may be found in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co., Easton, PA (1990). Suitable routes may include oral, rectal, transdermal, vaginal, transmucosal, or intestinal administration; parenteral delivery, including

intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections, just to name a few.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Use of pharmaceutically acceptable carriers to formulate the compounds herein disclosed for the practice of the invention into dosages suitable for systemic administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, the compositions of the present invention, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection.

The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the invention to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

Agents intended to be administered intracellularly may be administered using techniques well known to those of ordinary skill in the art. For example, such agents may be

encapsulated into liposomes, then administered as described above. Liposomes are spherical lipid bilayers with aqueous interiors. All molecules present in an aqueous solution at the time of liposome formation are incorporated into the aqueous interior. The liposomal contents are both protected from the external microenvironment and, because liposomes fuse with cell membranes, are efficiently delivered into the cell cytoplasm. Additionally, due to their hydrophobicity, small organic molecules may be directly administered intracellularly.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art. In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions. The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added.

All publications referenced are incorporated by reference herein, including the nucleic acid sequences and amino acid sequences listed in each publication. All the compounds disclosed and referred to in the publications mentioned above are incorporated by reference herein, including those compounds disclosed and referred to in articles cited by the publications mentioned above.

Other embodiments of this invention are disclosed in the following claims.

**WHAT IS CLAIMED IS:**

1. Method for treating obesity in a mammal, comprising the step of administering to said mammal a pharmaceutical composition comprising a compound which antagonizes the activity of PPARγ protein.

2. The method of claim 1, wherein said mammal is a human.

3. Method for treating obesity in a mammal, comprising the step of administering to said mammal a pharmaceutical composition comprising a compound which reduces the expression of PPARγ protein.

4. The method of claim 3, wherein said mammal is a human.

5. Method of screening for a compound for treating obesity, comprising the steps of:

providing an adipocyte cell;

contacting said compound with said adipocyte cell; and

measuring or detecting the level of triglyceride in said cell, wherein a significant decrease in said level comparing to the level before the contact of said compound with said cell is indicative of said compound being useful for treating obesity.

6. The method of claim 5, wherein said compound antagonizes the activity of PPARγ protein.

7. The method of claim 5, wherein said compound reduces the expression of PPARγ protein.

8. Method of screening for a compound for treating obesity, comprising the steps of:

providing a preadipocyte or adipocyte cell;

contacting said compound with said cell; and

measuring or detecting the level of lipoprotein lipase in said cell, wherein a significant decrease in said level comparing to the level before the contact of said compound with said cell is indicative of said compound being useful for treating obesity.

9. The method of claim 8, wherein said compound antagonizes the activity of PPARγ protein.

10. The method of claim 8, wherein said compound reduces the expression of PPARγ protein.

# INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/US 96/14909

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| E | WO 96 33724 A (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 31 October 1996<br>see page 19, line 15 - line 28; claim 22<br>--- | 1-4 |
| P,X | WO 96 23884 A (LIGAND PHARMACEUTICALS INCORPORATION) 8 August 1996<br>cited in the application<br>see abstract<br>see page 4, line 27 - page 5, line 22<br>see page 21, line 22 - page 22, line 15<br>see page 25, line 4 - line 20<br>see page 29, line 14 - line 26; claims 40,41<br>---<br>-/-- | 1-4 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 13 January 1997 | 06. 02. 97 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax (+ 31-70) 340-3016 | Hoff, P |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 96/14909

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| P,X | CHEMICAL ABSTRACTS, vol. 123, no. 21, 20 November 1995 Columbus, Ohio, US; abstract no. 281583, MOTOJIMA K.: "TOWARD THE TREATMENT OF OBESITY.ROLE OF PPAR GAMMA IN ADIPOGENESIS" XP002022572 see abstract & TANPAKUSHITSU KAKUSAN KOSO, vol. 40, no. 13, October 1995, pages 1936-1941, | 1-4 |
| Y | WO 95 18533 A (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) 13 July 1995 see abstract see page 16, line 35 - page 17, line 8; claims 2,5 | 1-4,6,7, 9,10 |
| Y | CELL, vol. 79, 1994, pages 1147-1156, XP000577080 P. TONTONOZ ET AL.: "STIMULATION OF ADIPOGENESIS IN FIBROBLASTS BY PPARgamma2, A LIPID-ACTIVATED TRANSCRIPTION FACTOR" cited in the application see the whole document | 1-4,6,7, 9,10 |
| Y | THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 22, June 1995, pages 12953-12956, XP000577082 J.M. LEHMANN ET AL.: "AN ANTIDIABETIC THIAZOLIDINEDIONE IS A HIGH AFFINITY LIGAND FOR PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR gamma" cited in the application see the whole document | 1-4,6,7, 9,10 |
| X<br>Y | US 3 884 758 A (GREEN) 20 May 1975<br>see the whole document | 5<br>6,7 |
| X<br><br><br>Y | WO 83 00930 A (THE ROCKEFELLER UNIVERSITY) 17 March 1983 see page 5, line 1 - line 17 see page 19, line 21 - line 32<br>see page 27, line 30 - page 33, line 7; claims 9,10 | 8<br><br><br>9,10 |

1

# INTERNATIONAL SEARCH REPORT

I national application No.
PCT/US 96/ 14909

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-4
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although claims 1-4 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/14909

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
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| WO-A-9623884 | 08-08-96 | AU-A- | 4863096 | 21-08-96 |
| WO-A-9518533 | 13-07-95 | NONE | | |
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| | | US-A- | 4822776 | 18-04-89 |
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